FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
112843-006	
U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	
09/674738	
INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE
PCT/EP98/04406	July 15, 1998
PRIORITY DATE CLAIMED	

TITLE OF INVENTION

USE OF BROMELAINE PROTEASES FOR INHIBITING BLOOD COAGULATION

APPLICANT(S) FOR DO/EO/US

Rainer MAURER; Klaus ECKERT; Edyta GRABOWSKA; and Klaus ESCHMANN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☒ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☒ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☐ Other items or information:

526 Rec'd PCT/PTO 03 NOV 2000

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/674738	PCT/EP98/04406	112843-005

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	**$1,000.00**
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	**$860.00**
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	**$710.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	**$690.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$860.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	12 - 20 =	0	x $18.00	**$0.00**	
Independent claims	2 - 3 =	0	x $78.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)** .			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$860.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)** .			☐	**$0.00**	
SUBTOTAL =				**$860.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$860.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED =				**$860.00**	
				Amount to be: refunded	$
				charged	$

☒ A check in the amount of **$860.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **02-1818** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Robert M. Barrett, Esq.
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Chicago, IL 60690-1135

SIGNATURE

Robert M. Barrett
NAME

30,142
REGISTRATION NUMBER

October 31, 2000
DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	Maurer et al.	DOCKET NO.:	112843-006
SERIAL NO:	09/674,738	ART UNIT:	Unknown
FILED:	October 31, 2000	EXAMINER:	Unknown
INVENTION:	"USE OF BROMELAIN PROTEASES FOR INHIBITING BLOOD COAGULATION"		

PRELIMINARY AMENDMENT

IN RESPONSE TO NOTICE OF MISSING REQUIREMENTS

Sir:

IN THE SPECIFICATION

Please insert the paper copy of the "Sequence Listing" entitled SEQUENCE LISTING (1 page) and attached herewith after page 11.

REMARKS

This Amendment is submitted in response to the Notice of Missing Requirements dated May 21, 2001. A copy of the Notice of Missing Requirements is attached herewith.

In the Notice of Missing Requirements, the Patent Office asserts that Applicants have not submitted the required sequence listing pursuant to 37 C.F.R. § 1.821-1.825.

In response, Applicants respectfully submit herewith a paper copy of the "Sequence Listing"; and a translation of the sequence listing entitled SEQUENCE LISTINGS (2 pages). Further, Applicants respectfully submit that a computer readable form of the "Sequence Listing" was submitted to the Patent Office, today, via EFS. Attached herewith is an acknowledgment receipt regarding same. Applicants state that the information recorded in computer readable form is identical to the written sequence listing that was filed herewith.

As previously discussed, Applicants have amended the Specification to direct entry therein of the paper copy of the "Sequence Listing."

Accordingly, Applicants respectfully submit that the requirements with respect to the Notice of Missing Requirements have been fully satisfied.

For the foregoing reasons, Applicants respectfully request an early and favorable examination of their patent application.

Respectfully submitted,

(Reg. No. 30,142)

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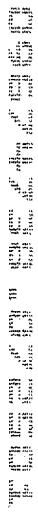
Acknowledgment Receipt:

APPLICATION NUMBER: **09674738**
FIRST NAMED INVENTOR: **Rainer Maurer**
TITLE OF INVENTION: **USE OF BROMELAIN PROTEASES FOR INHIBITING BLOOD COAGULATION**
ATTORNEY DOCKET NUMBER: **112843-006**

FILE LISTING:
tran00006.xml 6482 Bytes
BROMELA1.APP 1055 Bytes
00006bio.xml 1031 Bytes
u-bio.dtd 3619 Bytes
e-bioseq.xsl 6067 Bytes

EFS ID: **12175**
FILE SIZE: **16750 Bytes**
TIMESTAMP: **Tue Aug 21 12:36:46 EDT 2001**
MESSAGE DIGEST: **jcRlnMIyZtrGd/0b3Csn4A==**
DIGITAL CERTIFICATE HOLDER NAME: **cn=Robert M. Barrett, ou=Registered Attorneys**
UPLOAD STATUS: **You have successfully uploaded your submission to USPTO**



PCT/EP98/04406

Ursapharm Arzneimittel GmbH

Use of Bromelaine Proteases for Inhibiting Blood Coagulation

The present invention relates to the use of bromelaine proteases, preferably basic bromelaine proteases, notably for inhibiting the blood coagulation system, especially for stimulating the production of plasmin, for inhibiting the production of fibrin and for inhibiting the adhesion of human thrombocytes to endothelium cells.

Bromelaine is a mixture of quite different proteins that may be isolated from plants of the family Bromeliaceae, the exact composition of which could so far not yet be completely characterized due to the complexity and variety of the components contained therein. It could, however, be shown that bromelaine contains different phosphatases, cellulases, glycosidases, cysteine proteases and the peptide inhibitors thereof, as well as additional not yet more closely identified components. The material and quantitative composition of bromelaine, however, varies in response to the origin and the isolation procedure from the respective source, so that different methods for isolating the raw product, for standardizing the same as well as for purifying specific components contained therein, have been developed.

Some of the components in bromelaine have already been identified more closely. Thus, it is reported by Murachi et al. in The Journal of Biological Chemistry 1 (1960), 99-107, that bromelaine contains at least 5 similarly acting proteases with a different substrate specificity and a different pH optimum.

During studies performed with bromelaine it has, moreover, been found that said mixture can also be used as a medicament for treating different states of diseases.

Thus, DE 41 30 221 proposes the use of papain and/or trypsin, specific proteolytic enzymes derived from the bromelaine mixture, for the production of a medicament, which is to be suitable for treating autoimmune diseases. According to said patent, the papain, or the trypsin respectively acts on proteins participating in the development of autoimmune diseases, which comprise a C_H2-domain.

The use of bromelaine as a mixture for cancer therapy and/or metastasis prophylaxis is moreover disclosed in DE 43 02 060, in which it is assumed that bromelaine acts on CD44, a strongly glycosylized surface protein present on different cells of the organism, which is said to play a role in the development of tumors.

The isolation and characterization of a protease from the bromelaine mixture is explained in WO 95/00169, which acts on the synthetic pathway of cyclic nucleotides. The enzyme designated as "Stem Bromelaine Protease" comprises 213 amino acids and is to obviate diseases, such as the formation of tumors, atherosclerosis or bacterial infections.

Due to the development in the field of purification techniques it has been possible to isolate and partially also characterize additional components from the bromelaine mixture. Thus, it was disclosed by Eckert et al. in The Journal of Protein Chemistry **14** (1995), 41-52, that bromelaine contains at least 8 basic proteases, which could be fractioned by means of FPLC-cation exchange-chromatography. Also, the existence of two forms of acidic proteases could be shown (Maurer et al., Journal of Protein Chemistry **17** (1998), 351-361).

Although different medical fields of application for bromelaine have been found, there is a need to find additional applications for bromelaine. It would thereby be desirable, due to the not yet completely understood interactions of the individual components in the mixture, not to use the mixture itself in the respective field of application, but only the component of the mixture responsible for the respective purpose. A problem arises in this respect, however, as it cannot be predicted whether individual components are effective by themselves in an isolated state without other additional substances present in the bromelaine mixture, or

whether they rather require additional components present in the bromelaine mixture as auxiliary substances, which have so far not yet been identified.

It is an object of the invention to provide additional possibilities to use bromelaine, especially the components thereof.

Another object of the invention resides in identifying the component(s) responsible for the respective medical use, and in providing access thereof to a medical use.

The inventors have carried out extensive studies and have surprisingly found, that an inhibition of blood coagulation can be achieved solely with the proteases present in the bromelaine mixture, without the other components present in said mixture.

Consequently, the above-mentioned problem is solved by using the proteases present in the bromelaine mixture for inhibiting blood coagulation.

It has shown that especially the production of plasmin is stimulated by the bromelaine proteases, while the formation of fibrin and the adhesion of thrombocytes on endothelium cells – all of which are processes playing a significant role in blood coagulation – are inhibited.

In a preferred embodiment of the invention especially basic proteases are applied for the indicated purpose, preferably the bromelaine proteases obtained as fractions F4, F5 or, more preferably, F9 in accordance with the method described by Eckert et al. in the Journal of Protein Chemistry 14 (1995), 41-52.

The protease contained in fraction F4 has a molecular weight of about 24.4 KDa and an optimal activity at a pH in the range of about 4 to 5.5. The protease further comprises the following amino acid sequence:

Val Pro Gln Ser Ile Asp Trp Arg Asp Tyr

Gly Ala Val Thr Ser Val Lys Asn Gln Asn

The protease contained in fraction F5 has a molecular weight of about 24.5 KDa and an optimal activity at a pH in the range of about 3.5 to 5. The protease further comprises the following amino acid sequence:

Val Pro Gln Ser Ile Asp Trp Arg Asp Tyr
Gly Ala Val Thr Ser Val Lys Asn Gln Asn

The protease contained in fraction F9 has a molecular weight of about 23.4 KDa and an optimal activity at a pH in the range of about 6 to 8. The protease further comprises the following amino acid sequence:

Val Pro Gln Ser Ile Asp Trp Arg Asp Ser
Gly Ala Val Thr Ser Val Lys Asn Gln Gly

It has surprisingly shown that an effective inhibition of blood coagulation can be achieved by using bromelaine proteases, and that said inhibition can be obtained merely with the proteases isolated from said bromelaine mixture, without other additional components present in the bromelaine mixture playing a role.

The proteases can be administered to a subject in a manner already known in connection with the bromelaine mixture, i.e. by intravenous or intraperitoneal or preferably by oral administration, wherein the active substances are then formulated with excipients commonly used in the prior art, for passing the proteases through the gastrointestinal tract in an active form so as to guarantee a systemic availability.

The proteases can be isolated in accordance with conventional methods. Especially a purification as indicated by Eckert et al. in the Journal of Protein Chemistry 14 (1995), 41-52 and by Maurer et al. in the Journal of Protein Chemistry 17 (1998), can be applied. Upon purification, said proteases can be initially sequenced, and the corresponding gene can be

isolated from the genome of e.g. the pineapple by means of molecular-biological methods. By means of molecular-biological methods a recombinant protein can then be provided in a conventional manner.

The invention will now be explained in more detail by means of the following examples, which merely are explanatory and are not to be construed to limit the present invention.

The proteases used in the present invention, especially the basic proteases, are isolated according to Eckert et al., The Journal of Protein Chemistry 14 (1995), 41-52 and according to Maurer et al., The Journal of Protein Chemistry 17 (1998). The contents of said publications are herewith entirely included in the contents of disclosure of the present application.

As example of the effects of bromelaine proteases on blood coagulation, the fraction F9 isolated according to the above-mentioned documents will be used substitutionally.

Effects of bromelaine F9 on the fibrinolysis

For determining the effect, a method based on the use of a chromogenic substrate in a photometric system is applied. By means of the used test kit Berichrom-Pasminogen (Behring) the plasminogen of the sample is transferred into a complex by streptokinase. During the kinetic test, the release of plasmin can be detected in terms of quantity through the extinction increase by adding the plasmin substrate.

Example 1

In this experiment, the fibrinolytic activity of bromelaine F9, bromelaine base powder (raw product) and streptokinase is compared.

The starting material for determining the fibrinolytic activity of the protease bromelaine F9 to be tested is the citrate plasma of healthy donors. 9 parts of venous blood are mixed with 1 part of sodium citrate solution (0.11 mol/l) and are subsequently centrifuged for 10 min (1500 x g). Streptokinase, urokinase, tPA, plasmin substrate, the test substance bromelaine F9 as well as the plastic cuvettes are preheated to 37°C in an incubator. 20 ml of the plasma sample, 500 ml of the streptokinase (ready-to-use test kit solution), urokinase (1U/ml), tPA = Actilyse® (0.58 x 10^6 I.E./ml) or of the bromelaine F9 solution are pipetted into the measuring cuvette. Upon mixing, the solution is incubated for 5 min. at 37°C. The reaction is started by adding 100 ml of plasmin substrate (ready-to-use test kit solution). The extinction at 405 nm is measured in response to the concentration of the sample and time.

Table 1

Fibrinolytic activity of streptokinase, bromelaine F9 and bromelaine base powder in the plasminogen test

Time (s)	Streptokinase (kit)	Bromelain F9 (µg/ml)			Bromelaine Base Powder 50 µg/ml
		5	10	30	
30	0.284	0.23	0.315	0.304	0.356
60	0.523	0.424	0.485	0.559	0.449
120	0.741	0.610	0.611	0.795	0.507
180	1.078	0.929	0.929	1.036	0.551

As can be seen from table 1, bromemlaine F9 shows in the kinetic test an effect comparable to that of the streptokinase. The effect of bromelaine F9 is dependent on time and the concentration, the maximum effect is obtained at 30 mg/ml (1.0 U/mg). Already at a concentration of 5 mg/ml (E = 0.929) bromelaine F9 is superior to the effect of the

bromelaine base powder (0.4 U/mg) in a concentration of 50 mg/ml (E = 0.55).

Example 2

The objective of this experiment resides in testing whether and to what extent the combination of bromelaine F9 with streptokinase is superior to the effect of streptokinase alone.

Table 2

Fibrinolytic activity of streptokinase alone
and in combination with bromelaine F9 in the plasminogen test

Time (s)	Streptokinase (kit)	Streptokinase + Bromelaine F9
30	0.284	0.246
60	0.523	0.479
120	0.741	0.728
180	0.078	0.939

As can be seen from table 2, the combination of bromelaine F9 (10 mg/ml) with streptokinase in the plasminogen test is not superior to the effect of streptokinase alone.

This can be interpreted in that the effect of bromelaine F9 on the fibrinolysis (formation of plasmin) has a characterization similar to that of streptokinase, however, is 10 times higher (relative to the chemical concentration) than that of bromelaine base powder. The effect of bromelaine F9 is dependent on the concentration and time. The kinetics correspond to those of streptokinase alone in said system.

Example 3

In this experiment, the fibrinolytic activities of urokinase, tissue plasminogen activator (tPA) and the combinations thereof are compared to that of bromelaine F9.

Table 3

Fibrinolytic activity of urokinase, tPA alone and the combination with bromelaine F9 in the plasminogen test

Time (s)	Urokinase (1U/ml)	TPA $0.58x10^6$ I.E./ml	**Urokinase +** Bromelaine F9 (10µg/ml)	tPA + Bromelaine F9 (10µg/ml)
30	0.2216	0.2315	0.2757	0.2417
60	0.3517	0.3215	0.3888	0.3124
120	0.5830	0.4469	0.5244	0.4680
180	0.7970	0.7899	0.6640	0.7553

As can be seen from the comparison of the values illustrated in table 1 and 3, the streptokinase in this test system effects a stronger plasminogen conversion in contrast to urokinase and tPA. The effect of 30 mg/ml bromelaine F9 (tables 1, 3) corresponds to the effect of streptokinase and is superior to the effect of bromelaine base powder. In a combination of bromelaine F9 with the above-mentioned plasminogen activators, no stronger effects can be shown in contrast to the sole effect of urokinase and tPA, or of streptokinase (table 2).

Effect of bromelaine F9 on the production of fibrin from human plasma of healthy donors

In this connection it is the objective to test whether and to what extent bromelaine F9 influences the thrombin-induced production of fibrin from human plasma.

Example 4

The starting material is citrate plasma of healthy donors, which is pre-incubated with bromelaine F9 at 37°C and is mixed with thrombin afterwards. Per test 0.02 ml protease solution are pipetted to 0.05 ml citrate plasma and are incubated for 1 hour. Next, 0.01 ml thrombin (0.2 U/ml) are added and an incubation of 10 min. in the water bath takes place at 37°C. The production of fibrin is evaluated semi-quantitatively, organoleptically under the invert microscope (twenty-fold enlargement).

It is found thereby, that bromelaine F9 (100 mg/ml) just like streptokinase, completely prevents the thrombin-induced production of fibrin from citrate plasma. On the basis of the applied chemical concentration bromelaine F9 is more effective than bromelaine base powder by the factor 2. In contrast thereto, papain (100 mg/ml, specific activity 7.1 U/mg) has no effect under these conditions.

Effect of bromelaine F9 on the adhesion of human thrombocytes to BKEz-7 bovine endothelium cells

Thrombocytes isolated from human whole blood are marked with the fluorescence dye 2,7-bis-(2-carboxyethyl)-5,6-carboxyfluoresceinacetoxymethylester. Permanent BKEz-7 bovine aorta cells (11th-22nd passage) are pipetted into a 96 microtiter plate with 60,000 cells per recess and are incubated over night. For the thrombocytes-endothelium cell-adhesion-assay $5x10^7$ thrombocytes after an incubation time of 15 min. at 37°C are optimal. The removal of the non-bonded thrombocytes is effected by washing the cells with KRB-buffer (Krebs-Ringer-bicarbonate buffer with 5.6 mMol Glucose + 1 % BSA) twice.

Example 5

It is tested in said experiment as to which effect bromelaine F9 has on already adherent thrombocytes. After performance of the thrombocytes-endothelium cell-adhesion-assay the adherent thrombocytes (stimulated with 0.2 U/ml thrombin) are incubated with bromelaine F9 (0.01 mg/ml) for 10 min. at 37°C. As a control, bromelaine base powder (0.1 mg/ml) is tested as well. The resulting thrombocytes bonds on the endothelium cells are compared with those of the samples not treated with protease. As can be seen from table 4, bromelaine F9 reduces the bonding of thrombocytes by 32 % (68 % bonding), while bromelaine base powder becomes effective only at a concentration of 0.1 mg/ml, with a reduction of the thrombocytes bonding by 40 % (60 % bonding).

Table 4

Adhesion of thrombocytes on BKEz-7 endothelium cells
under the influence of bromelaine F9

- Thrombin	+ Thrombin (0.2 U/ml)	+ Bromelaine F9 (0.01 µg/ml)	+ Bromelaine Base Powder (0.1 µg/ml)
% Adhesion			
61*	100	68*	60*

The measured fluorescence intensities of the thrombin-stimulated adhered thrombocytes are standardized to 100 %;

* $p < 0.001$ (t-test); in contrast to the adherent, thrombin-stimulated thrombocytes, said differences are statistically significant.

Example 6

Isolated human thrombocytes ($5x10^7$/ml) are incubated with bromelaine F9 and bromelaine base powder in different concentrations for 15 min. at room temperature, the proteases are removed by centrifugation (1000 x g) and washing, the thrombocytes are resuspended in 1 ml KRB buffer (see above), incubated with 0.2 U/ml of thrombin and used in the adhesion assay on the BKEz-7 cells. The results are illustrated in table 5.

Table 5

Adhesion of thrombocytes on BKEz-7 endothelium cells
under the influence of bromelaine F9 and Bromelaine Base Powder

- Thrombin	+ Thrombin (0.2 U/ml)	+ Bromelaine F9 (µg/ml)		+ Bromelaine Base Powder (0.1 µg/ml)
		0.005	0.01	
% Adhesion				
61*	100	86*	75*	69*

* $p < 0.001$ (t-test); in contrast to the adherent, thrombin-stimulated thrombocytes, said differences are statistically significant.

As can be seen from table 5, bromelaine F9 shows a concentration-dependent inhibition of the adhesion of the thrombocytes on the endothelium cells. A small reduction of adhesion of the thrombocytes is determined for bromelaine base powder in a concentration of 0.1 mg/ml.

PCT/EP98/04406
Ursapharm Arzneimittel GmbH

Amended claims as attached to the IPER

Patent Claims

1. Use of bromelaine proteases for inhibiting blood coagulation, wherein the bromelaine proteases are selected from the group consisting of:

a) a basic bromelaine protease, having
- a molecular weight of about 24.4 KDa,
- an optimal activity at a pH in the range of 4 to 5.5, and
- comprising the following amino acid sequence:

Val Pro Gln Ser Ile Asp Trp Arg Asp Tyr
Gly Ala Val Thr Ser Val Lys Asn Gln Asn

and/or

b) a basic bromelaine protease, having
- a molecular weight of about 24.5 KDa,
- an optimal activity at a pH in the range of 3.5 to 5, and
- comprising the following amino acid sequence:

Val Pro Gln Ser Ile Asp Trp Arg Asp Tyr
Gly Ala Val Thr Ser Val Lys Asn Gln Asn

and/or

c) a basic bromelaine protease, having
- a molecular weight of about 23.4 KDa,
- an optimal activity at a pH in the range of 6 to 8, and
- comprising the following amino acid sequence:

Val Pro Gln Ser Ile Asp Trp Arg Asp Ser
Gly Ala Val Thr Ser Val Lys Asn Gln Gly.

2. Use according to claim 1, wherein the plasmin production is stimulated.

3. Use according to claim 1, wherein the production of fibrin is inhibited.

4. Use according to claim 1, wherein the adhesion of thrombocytes on endothelium cells is inhibited.

5. Medicament for inhibiting blood coagulation, wherein the medicament, apart from conventional excipients and auxiliary substances, consists of one or more bromelaine proteases according to claim 1.

6. Medicament according to claim 5, wherein the bromelaine protease is a recombinant bromelaine protease.

Docket No.
112843-006

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
USE OF BROMELAINE PROTEASES FOR INHIBITING BLOOD

☐ the specification of which

(check one)

☐ is attached hereto.

☒ was filed on **October 31, 2000** as United States Application No. or PCT International Application Number **09/674,738**

and was amended on _____

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
PCT/EP98/04406	**PCT**	**15 July 1998**	☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	
			☐
(Number)	(Country)	(Day/Month/Year Filed)	

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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3rd

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Fifth inventor's signature	Date
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SEQUENCE LISTING

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<120> Use of Bromelaine Proteases for Inhibiting Blood Coagulation

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<140> US 09/674,738
<141> 2000-11-03

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INFORMATION FOR SEQ ID NO: 2:

SEQUENCE CHARACTERISTICS:
LENGTH: 20
TYPE: amino acids
STRANDEDNESS: single strand
TOPOLOGY: linear

INITIAL ORIGIN:
ORGANISM: pineapple (Bromeliacea)

SEQUENCE DESCRIPTION:

Val Pro Gln Ser Ile Asp Trp Arg Asp Ser Gly Ala Val Thr Ser Val
1 5 10 15

Lys Asn Gln Gly
20

SEQUENCE LISTINGS

GENERAL INFORMATION:

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ZIP CODE: 66129

TITLE OF THE INVENTION: Use of Bromelaine Proteases for Inhibiting Blood Coagulation

NUMBER OF SEQUENCES: 2
COMPUTER READABLE FORM:
DATA CARRIER: diskette
COMPUTER: IBM-PC-Compatible
OPERATING SYSTEM: MS DOS

INFORMATION FOR SEQ ID NO: 1:

SEQUENCE CHARACTERISTICS:
LENGTH: 20
TYPE: amino acids
STRANDEDNESS: single strand
TOPOLOGY: linear

INITIAL ORIGIN:
ORGANISM: pineapple (Bromeliacea)

SEQUENCE DESCRIPTION:

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